Final Scientific Report

Cover Page

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Project Title: Potentiation of pest control by insect immunosuppression

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Collaborating Investigators:	

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order**:**

Budget: IS: $ 150000 US: $ 150000 Total: $ 300000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1	1		2
Submitted, in review, in preparation	2			2
Invited review papers				
Book chapters				
Books				
Master theses			1	1
Ph.D. theses		1	1	2
Abstracts	3			3
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Baochun Li – position number 50123244

3 graduate students also received partial support from this grant

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1		2
Longer Visits (Sabbaticals)				

Description Cooperation:

The PIs communicated regularly be email and telephone and exchanged visits over the course of this project. Research materials and data was exchanged through these communications and used to design subsequent experiments and manuscripts. The complementary expertise of the investigators in baculoviruses (Chejanosvky) and polydnaviruses (Webb) proved useful in the investigation of the functional effects of polydnavirus genes and their synergistic potential in the baculovirus system.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

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Abstract

The restricted host range of many baculoviruses, highly pathogenic to Lepidoptera and non-pathogenic to mammals, limits their use to single or few closely related Lepidopteran species and is an obstacle to extending their implementation for pest control. The insect immune response is a major determinant of the ability of an insect pathogen to efficiently multiply and propagate. We have developed an original model system to study the Lepidopteran antiviral immune response based on *Spodoptera littoralis* resistance to AcMNPV (*Autographa californica* multiple nucleopolyhedrovirus) infection and the fascinating immunosuppressive activity of polydnaviruses . Our aim is to elucidate the mechanisms through which the immunosuppressive insect polydnaviruses promote replication of pathogenic baculoviruses in lepidopteran hosts that are mildly or non-permissive to virus- replication. In this study we :
1- Assessed the extent to which and the mechanisms whereby the immunosuppressive *Campoletis sonorensis* polydnavirus (CsV) or its genes enhanced replication of a well-characterized pathogenic baculovirus AcMNPV, in polydnavirus-immunosuppressed *H. zea* and *S. littoralis* insects and *S. littoralis* cells, hosts that are mildly or non-permissive to AcMNPV.
2- Identified CsV genes involved in the above immunosuppression (e.g. inhibiting cellular encapsulation and disrupting humoral immunity).
We showed that:
1. *S. littoralis* larvae mount an immune response against a baculovirus infection.
2. Immunosuppression of an insect pest improves the ability of a viral pathogen, the baculovirus AcMNPV, to infect the pest.
3. For the first time two PDV-specific genes of the vankyrin and cystein rich-motif families involved in immunosuppression of the host, namely Pvank1 and Hv1.1 respectively, enhanced the efficacy of an insect pathogen toward a semipermissive pest.
4. Pvank1 inhibits apoptosis of Spodopteran cells elucidating one functional aspect of PDV vankyrins.
5. That Pvank-1 and Hv1.1 do not show cooperative effect in *S. littoralis* when co-expressed during AcMNPV infection.

Our results pave the way to developing novel means for pest control, including baculoviruses, that rely upon suppressing host immune systems by strategically weakening insect defenses to improve pathogen (i.e. biocontrol agent) infection and virulence. Also, we expect that the above result will help to develop systems for enhanced insect control that may ultimately help to reduce transmission of insect vectored diseases of humans, animals and plants as well as provide mechanisms for suppression of insect populations that damage crop plants by direct feeding.

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Achievements

1. Polydnavirus genes that immunosuppress S. littoralis larvae sustain efficient baculovirus infection via the oral route.

During this project we demonstrated that immunosuppression of *S. littoralis* either by parasitization with *Chelonus inanitus* wasp (that delivered CiV) or by targeted expression of *Campoletis sonorensis* polydnavirus genes from the *vankyrin* or the *cystein rich-motif* families, rendered the larvae highly susceptible to AcMNPV infection via the haemocoelic route (1). However, in nature *S. littoralis* contract the baculovirus infection by ingesting the viral polyhedral particles dispersed in the leaves and soil. To study the impact of immunosuppressing the insect on the oral baculoviral infection we constructed the polyhedra-positive baculoviruses vPvank1pol and Hv1.1pol bearing the Pvank1 and Hv1.1 genes from the *vankyrin* and *cystein rich-motif* families, respectively. In these constructs expression of the latter genes was directed by the *Drosophila* hsp70 promoter. Wild type AcMNPV and vHSPGFP (a polyhedra-positive AcMNPV-recombinant bearing GFP controlled by the *Drosophila* hsp70 promoter) served as control viruses (Fig.1, Appendix). We assayed the susceptibility of neonates, 1st to 4th instars of *S. littoralis* to viral infection, since it is well documented that it is higher at earlier stages of insect development. Infections were performed by the drop- or diet- feeding methods. 4th instar larva were also infected by oral inoculation as well to deliver exacts amounts of polyhedra to the insect gut. vHv1.1 required lower doses than wild type AcMNPV to cause mortality of the larvae and when administrated at the same doses it caused about 2-fold increase in larval mortality (compare for example mortality of larvae infected at 10,000 PIB with vHv1.1 and AcMNPV in Fig. 2). On the other hand, vPvank1 caused mortality rates similar to AcMNPV (not shown). When the time to death was examined both, vPvank1 and vHv1.1, were faster to kill the insects than AcMNPV (Fig. 3). Thus, as estimated by graphic extrapolation, the LT_{50} values of vHv1.1 were about 5 days compared to 6.5 days for AcMNPV for *S. littoralis* 1st instar larvae and, of 5 days *vs* 6 days for Pvank1 *vs* AcMNPV infected neonate larvae, respectively.

We concluded that Pvank1 and Hv1.1-mediated immunosuppression of *S. littoralis* enhanced the oral infectivity of AcMNPV.

Further, we engineered baculoviruses expressing *gfp*-tagged versions of Pvank1 and Hv1.1 (Fig. 1). These viruses displayed similar infectivity values than their wild type counterparts (vHv1.1 and vPvank1, not shown). Close examination of the midgut of *S. littoralis* larvae

orally infected with vPvank1-GFP and vHv1.1-GFP showed enlarged foci compared to larvae infected with vHSGFP (compare Fig. 4B and 4C, to 4A) indicating that expression of the immunosuppressive Pvan1 and Hv1.1 facilitated the dissemination of the baculovirus to the neighboring cells by escaping encapsulation (Fig. 4D and E).

2. Concerted expression of Hv1.1 and Pvank1

Since Hv1.1 and Pvank1 are co-expressed in larvae parasitized by *C. sonorensis* that inject the polydnavirus CsIV, we analyzed the impact of their co-expression on baculovirus-infected *S. littoralis*. For that purpose we orally administered equal amounts of vHv1.1 and vPvank1 polyhedra to *S. littoralis* 2nd instar larvae. Simultaneous administration of the above viruses did not result in enhanced mortality compared to their individual administration (not shown).

3. The roles of NF-κβ, Iκβ, and vankyrin interactions in insect antimicrobial and antiviral responses

The vankyrin genes are so-named because of their sequence similarity to genes inhibiting the *NF-κβ* signaling pathway, the *Iκβ* genes. There are seven *Iκβ* genes in CsIV some of which are preferentially expressed in fat body and others in hemocytes. All have been expressed in transient and transformed cell assays and shown to respond to immune challenge with a shift in their subcellular localization (3). We suspect that divergent *vankyrin* gene family members target different NF-κβ dimers in different tissues (i.e. fat body vs. hemocytes).

We noted that cells expressing the vankyrin genes that are preferentially expressed in the fat body (Pvank1 and I2vank3) exhibited prolonged viability after infection with a baculovirus (2). This was an intriguing result because *NF-κβ* signaling pathways are known to interact with the cellular apoptotic response and baculoviruses are known to express anti-apoptotic genes. So if the vankyrin genes were inhibiting apoptosis to prolong viability of cells infected with baculoviruses it must be acting at a different step in the apoptotic pathway. We have pursued this observation by assessing the extent to which cells induced to undergo apoptosis with a chemical agent were protected by expression of several vankyrin genes. Interestingly expression of any vankyrin gene tested inhibited chemically induced apoptosis with this effect most pronounced in cells expressing the fat-body specific vankyrin genes (Pvank1, and I2vank3, Fig. 5). This clear effect is now being pursued by investigating the features of the vankyrin genes through mutational analyses.

Concluding: The difference in susceptibility of 4th instar larvae to the injected recombinant baculovirus [100 % mortality at 139 hours, (1)] and its resistance to oral infection indicates that

the antiviral reaction observed in the midgut is still a major block to the viral infection. However in lower instars the dose-efficiency of the virus has been improved in the vHv1.1 *cys-motif* virus that also caused larger foci. Thus, the above results indicate that Hv1.1 enhances the efficacy of AcMNPV by facilitating accessibility of the virions to the internal organs of the larvae, hence requiring less infectious units to achieve higher mortality and probably, by enhancing the primary infection at the midgut level (local immunity). While vPvank1 that showed its best performance regarding the time-to-kill, may achieve a better transit of the virus via the tracheal system inhibiting tracheoblast melanization, probably triggered by the fat bodies' release of antiviral signals to mount a systemic infection, and also may act by suppressing fat body apoptosis (systemic immunity).

Our results pave the way to developing novel means for pest control, including baculoviruses, that rely upon suppressing host immune systems by strategically weakening insect defenses to improve pathogen (i.e. biocontrol agent) infection and virulence. Also, we expect that the above result will help to develop systems for enhanced insect control that may ultimately help to reduce transmission of insect vectored diseases of humans, animals and plants as well as provide mechanisms for suppression of insect populations that damage crop plants by direct feeding.

Our findings open up the possibility to perform a more detail analysis in the forthcoming years of the molecular mechanisms involved in manipulation of the insect host by the above viruses, including evaluation of the ability of *vankyrin* genes to inhibit apoptosis in the fat body and hemocytes of the baculovirus-infected larvae.

Description of the cooperation

We developed our collaboration through a synergistic cooperation: since the BW's lab mainly focuses in polydnaviruses and the Israeli lab in baculoviruses. NC visited BW's lab at the beginning of the project to coordinate its development and BW visited NC lab to discuss the achievements of the study and an outline of a continuation proposal. We evaluated progress made and discussed the application of new functional assays to elucidate the molecular mechanism of the *vank* and *cys-motif* genes immunosuppressed hosts. We also exchanged materials, expertise and data obtained throughout the first phase of the research project, Polydnaviruses, recombinant baculoviruses, antibodies and cell lines were exchanged. Host lepidopteran insects were not exchanged because they are not indigenous to both locations.

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List of Publications:

1. Rivkin, H., Kroemer, J. A., Bronshtein A., Belausov E., Webb B. A. and Chejanovsky N. Response of immunocompetent and immunosuppressed *Spodoptera littoralis* larvae to baculovirus infection (2006*). J. Gen. Virol.* 87, 2217–2.

2. Fath Goodin A., Kroemer J.A. and Webb B.A. (2009) The *Campoletis sonorensis* ichnovirus protein P-vank-1 inhibits apoptosis in insect Sf9 cells. (Insect biochemistry, accepted)

3. Fath-Goodin A., Nusuwardani T., Chejanovsky N., and Webb B.A., Functional dissection of the Campoletis sonorensis vankyrin gene family (in preparation).

4. Rivkin, H., Ornan, I., Bronshtein A., Belausov E., Webb B. A. and Chejanovsky N. Enhancement of baculovirus infectivity to *Spodoptera littoralis* by targeted immunosuppression with poydnavirus genes (in preparation).

Appendix

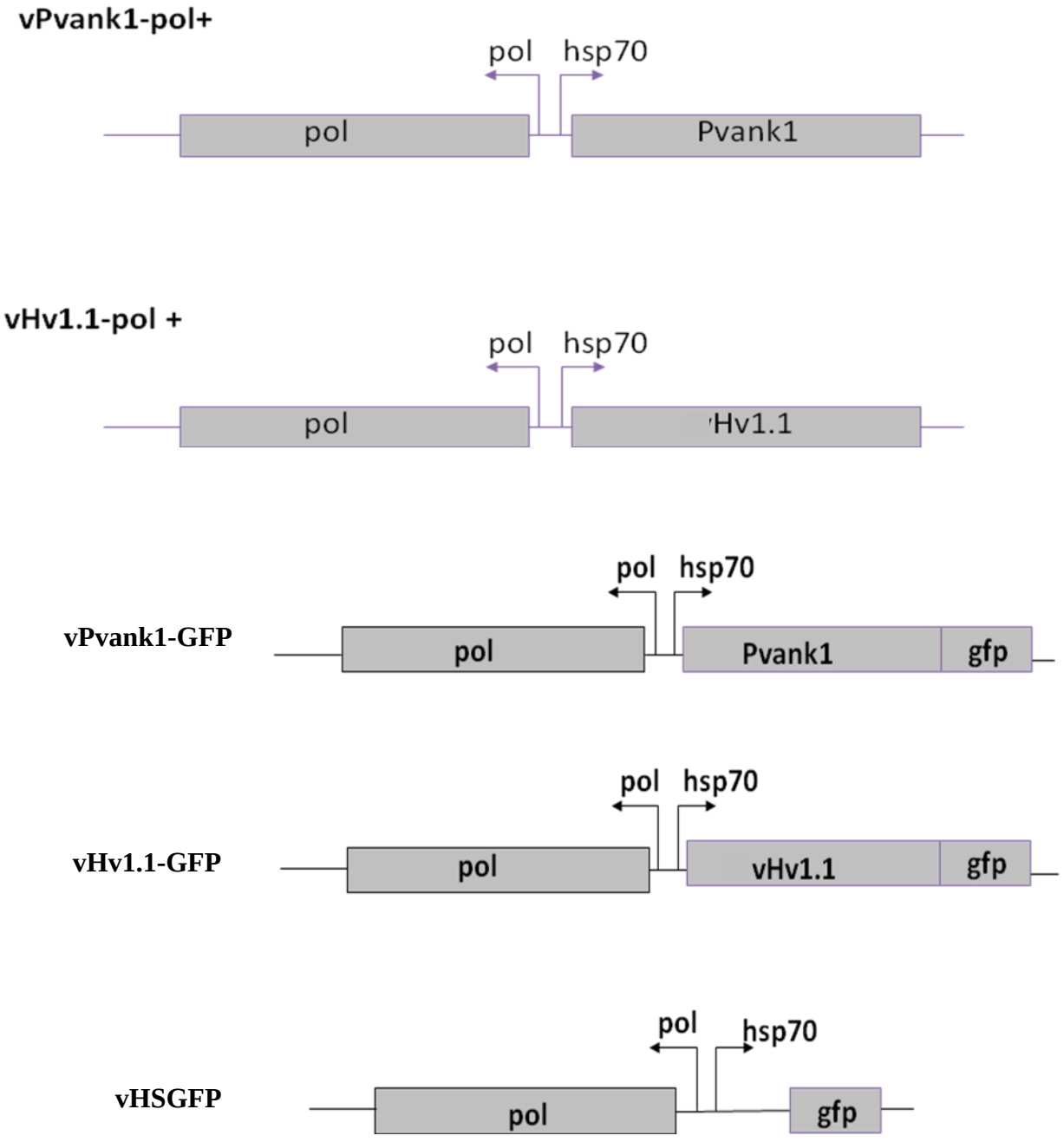

Fig.1 . Scheme of the polyhedrin locus of the viral constructs used in the study. pol: polyhedrin, gfp: green fluorescent protein . Pvank1 and Hv1.1 polydnavirus *vank* and *cys* motif genes, respectively.

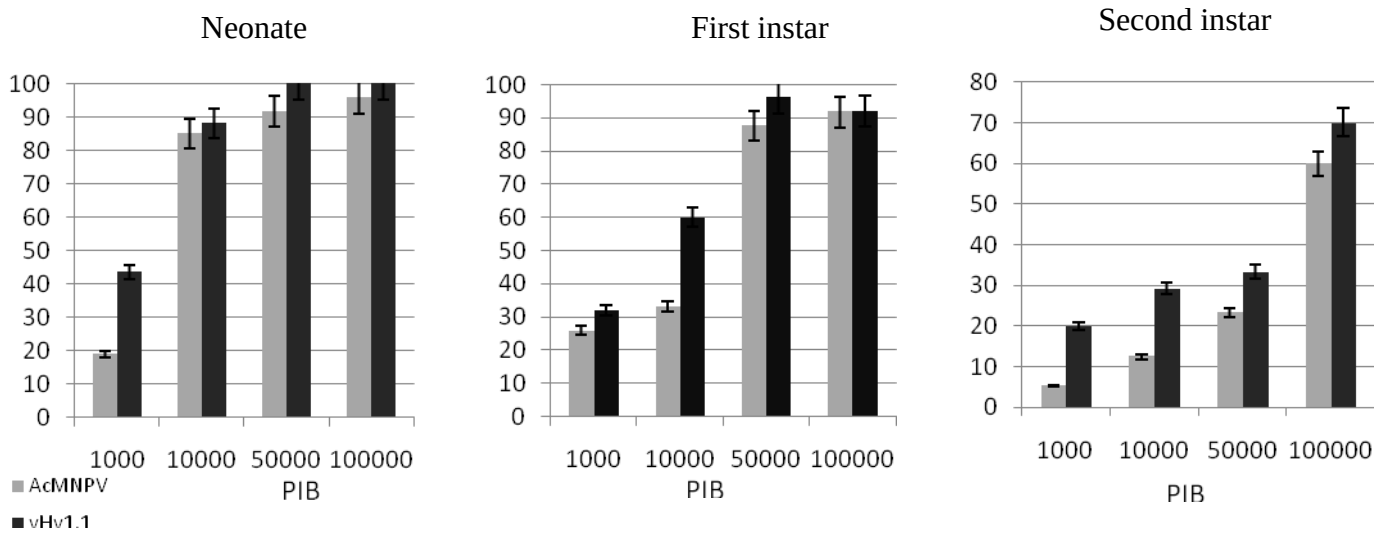

Fig.2 . Dose-response of *S. littoralis* larvae infected with recombinant baculoviruses expressing the polydnavirus immunosuppressive gene Hv1.. Neonate (less than 12 h old), 1st and 2nd instar larvae were orally fed infected with various doses of polyhedral inclusion bodies (PIBs) delivered by the droplet (neonates) or diet feeding assay (1st and 2nd instars). The experiments were repeated in triplicates.

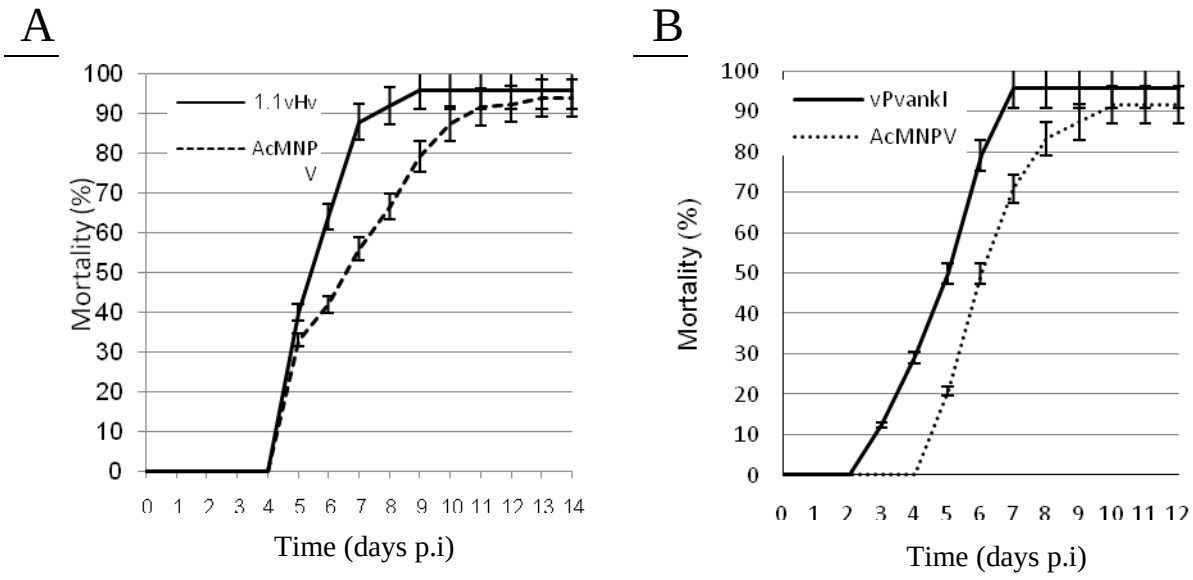

Fig. 3 . Time-response of *S. littoralis* larvae infected with recombinant baculoviruses expressing the polydnavirus immunosuppressive gene Hv1.1 and Pvank1. 1st instar (A) and neonate (B) larvae were fed infected LD_{95} doses of polyhedral inclusion bodies (PIBs) delivered by the droplet (neonates) or diet feeding methods (1st instars). The experiments were repeated in triplicates.

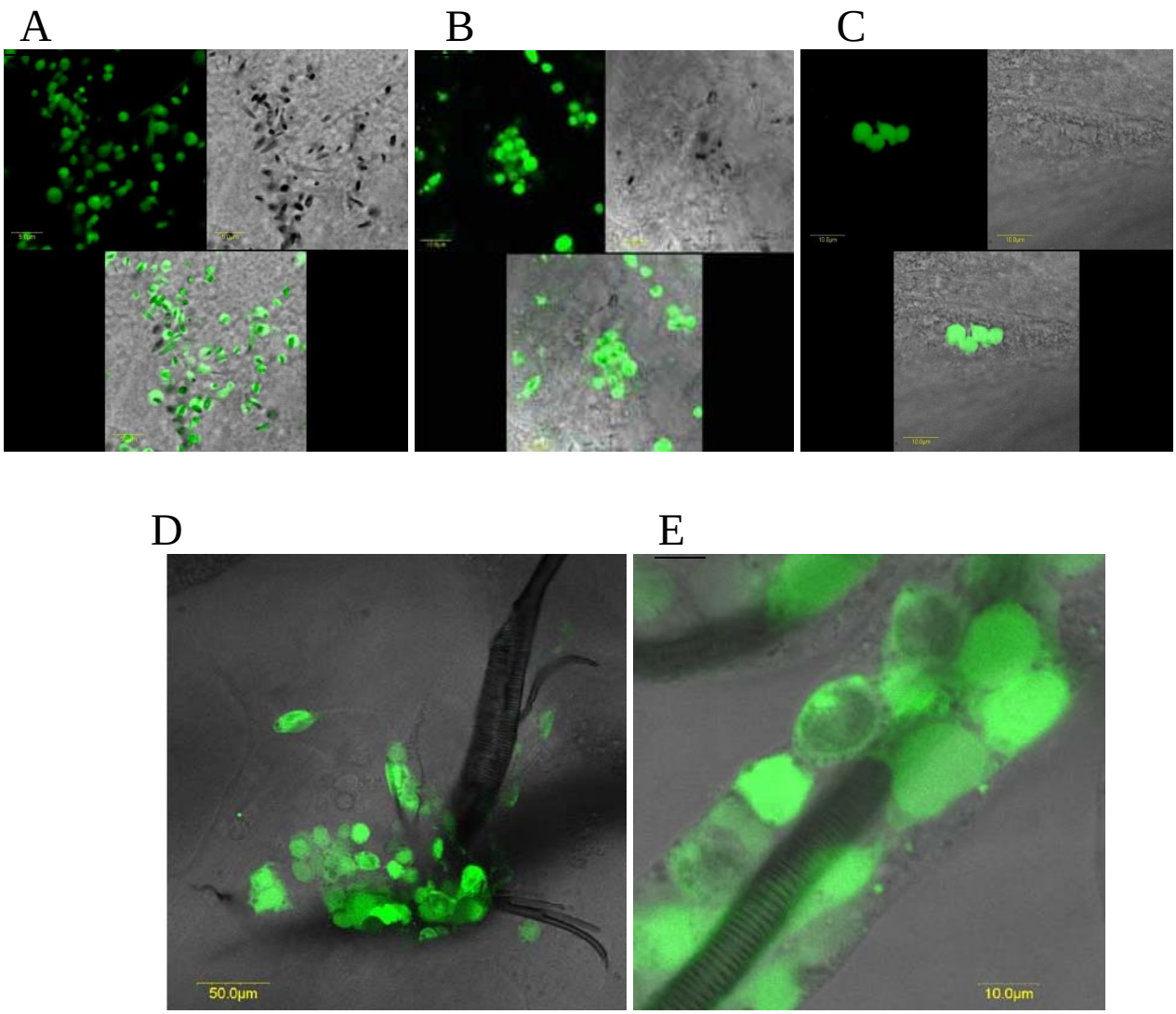

Fig. 4. *Impact of expressing Pvank1 and Hv1.1 on the propagation of AcMNPV in the body of S. littoralis*

Pathway of the infection in *S. littoralis* larvae orally infected with vHSPGFP (A), vPvank1GFPpol (B,E,F) and vHv1.1GFPpol (C). Barrs in A-C: . A- massive encapsulation of the wild type virus. B and C, enlarged foci. D and F, spread of the infection: access to the tracheal branches via the tracheoblasts.

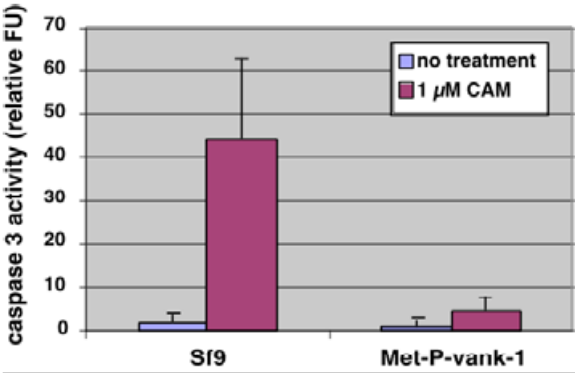

Fig. 5. P-vank-1 inhibits caspase-3 activity after induction of apoptosis in Sf9 cells. Apoptosis was induced in control Sf9 cells and Sf9 cells stably expressing P-vank-1 (Met-P-vank-1 cell line) with 1 μM CAM.Caspase-3 activity was determined using Ac-DEVD-AMC as afluorescent substrate at 20 h. For enzyme assay 3x105 cells were usedas described in Materials and Methods. Control cells were not CAMtreated.